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Biochemical Study of Mixed Culture Prototype in a Closed Ecological System

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BIOCHEMICAL STUDY OF MIXED CULTURE PROTOTYPE IN A CLOSED ECOLOGICAL SYSTEM

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SUMMARY

An investigation has been made to determine the ability of certain bacterial species to utilize various compounds associated with human urine as their main carbon and nitrogen source. The study was concerned primarily with growth and respiration of bacteria where citric or lactic acid served as the sole source of carbon and with the utilization of urinary urea in growing cultures.

The results show that of the four bacterial species tested for urease activity only Proteus vulgaris gave a positive reaction. Furthermore, this organism was able to convert from 94.72 to 98.46% of the urea of urine to ammonia and/or microbial protoplasm. The conversion of urinary urea by growing cultures of A. aerogenes and Serratia indica was considerable less than that observed for P. vulgaris.

The percent urea conversion for A. aerogenes and S. indica ranged from 7.79 to 37.10 and 16.89 to 22.01 respectively.

A great deal of variability was noted in the percent reduction in amount of urea by growing cultures of A. aerogenes and S. indica in relation to duplicate cultures within trials. This variability has been attributed to the variable characteristics of the different batches of urine employed in the experiment.

The data indicated that at least one of the test organisms studied in relation to urea utilization can be employed successfully in the biological conversion of urinary urea.

A study of growing cultures of selected bacteria has revealed that citric and lactic acids can supply the sole carbon-energy needs. The growth of organisms on a substrate containing either citrate or lactate as the sole carbon-energy source can be enhanced considerably when ammonium chloride serves as the nitrogen source. Urea, however, was also observed to serve as an excellent source of

nitrogen in growing cultures when citrate or lactate supplied the sole source of carbon-energy. The urea appeared to be somewhat inferior to ammonium chloride in supporting growth of the test organisms.

The results have demonstrated clearly that the citric and lactic acids of human urine can serve as the sole source of carbon and energy for selected bacterial species.

Respiration studies have been made with the various bacterial species heretofore mentioned in an effort to lend support to the growth studies already described in relation to the oxidation of citric and lactic acid.

The data indicated that citric acid cannot be oxidized by the test organism employed. Since the organisms were able to grow on citric acid as the sole source of carbon and energy, then this inability to oxidize the substrate has been attributed to impermeability of the cell wall of the organisms toward citric acid.

Lactic acid was observed to be oxidized by all the organisms used. The enzymes concerned with lactic acid oxidation are adaptative in nature.

Serratia indica was observed to oxidize lactic acid extensively in most of the manometric runs made; however, in some runs a short lag period was apparent.

The results of urea utilization, growth and respiration studies have clearly indicated that the urea content of urine can be depleted, the citric and lactic acids can be utilized as sole source of carbon and energy and that P. vulgaris and S. indica may be considered for future and extended studies of space biology.

Further investigations have been made on the biochemical nature of algal polysaccharides, carbonhydrate partition and availability of algae, utilization of algal end-products by yeast, and the conversion of detergent-like compounds to energy sources.

Algal polysaccharides (extracellular) were shown to contain various monosaccharides which can be utilized by man and other organisms. Glucose, galactose,

xylose, arabinose, fucose, rhamnose, and glucuronic acid were the sugars found. The compositional pattern of the polysaccharides varied with the species of algae.

With Palmellococcus, the extracellular polysaccharide contained twice as much glucose as any of the other constitutive monosaccharides. The molar ratio of glucose:arabinose:fucose:glucurone was shown to be 11:3:6:1. Glucose being the sugar in highest concentration is very important because of the readily available nature of this monosaccharide.

Studies on the biosynthesis of extracellular polysaccharides of green algae have shown that when a labeled hexose is added to an algal suspension, the extracellular polysaccharide is labeled much faster than when labeled bicarbonate is used. Also, the production was shown to be maximum at the optimum growth stage. The extracellular polysaccharide seems to be the same as the water-soluble, intracellular polysaccharide and cellular polysaccharide.

The substitution of various carbon sources for CO_2 produced variations in the extracellular polysaccharides in amounts and types of monosaccharides present. Glucose and lactate stimulated the formation of extra sugars detectable in the polysaccharides. The effects of other nitrogen sources besides KNO_3 on polysaccharide production by Palmellococcus were: calcium nitrate the best, ammonium nitrate, urea, sodium nitrate, and potassium nitrate in descending order. This effect was not the same as dry weight responses where only calcium nitrate and urea were above potassium nitrate.

Experimental results were obtained which demonstrated that Palmellococcus can be cultivated on waste substrates and that the substrates are enriched to a small extent with extracellular organics of algal origin.

Algal polysaccharides were shown to be oxidized to a degree by yeast. The best responses were received with the hydrolyzed polysaccharides. Algal extracts were shown to be readily oxidized by a yeast for energy. The growth response by

T. utilis to algal extracts was lower than the oxidative responses.

A carbohydrate analysis of Ch. vulgaris demonstrated that if energy is to be supplied wholly by algal carbohydrate, a method for cultivating large numbers of cells in a small culture system is needed.

Results on the conversions of detergent-like compounds to carbohydrate were very encouraging. Sodium lauryl sulfate and Tide were converted to a polysaccharide containing glucose, arabinose, ribose, and glucuronic acid. This polysaccharide was readily oxidized by T. utilis in the unhydrolyzed form.

INTRODUCTION

This progress report covers tests designed to ascertian the utility of human urine to support microbial growth intended for human consumption in outer space.

Experiments have been designed to study the following occurrences; (a) the utilization of urinary urea by four different species of bacteria, (b) respiration of various bacterial species on individual components commonly associated with urine and (c) growth of selected bacterial species on urine and individual components found in urine when supplied as the sole source of energy and carbon.

The role of algae in closed ecological systems has been further studied since the last progress report, the aim still being to evaluate the role of the algal cell in supplying energy from light and man's respiratory carbon dioxide to other microbial systems making up the closed ecology.

The research on algal polysaccharides has been continued. Data are presented in this report on: the carbohydrate composition of algal polysaccharides studied, factors affecting the production of extracellular polysaccharide by a selected species of algae, production of extracellular polysaccharides by a species of algae on waste substrates, studies on the biosynthesis of algal polysaccharides, and the response of a selected species of yeast to the extracellular polysaccharides of the test alga.

A complete carbohydrate analysis of Chlorella vulgaris with energy calculations is presented to express the form of potential algal energy supply and amount.

The feasibility of utilizing algal extract as carbon-energy sources for a selected species of yeast has been investigated.

The utilization of detergents and the conversion of detergents to carbohydrate material has been explored for possible use in a closed ecology designed for space travel.

PART I

METHODS AND PROCEDURES

I. Urinary Urea:

The reduction in the urea content of urine cultures was measured by the colorimetric procedure described by Ormsby, 1942. The analysis for urea was made at various intervals of incubation.

The urine used for the culture substrate was filter sterilized and inoculated with a washed cell suspension of the test organisms. Incubation was at 25 or 32°C.

The test organisms employed were: Aerobacter aerogenes, Proteus vulgaris Pseudomonas aeruginosa and Serratia indica.

II. Growth of Selected Bacterial Species on Various Components Associated with Urine.

The relative growth of bacteria was studied using media consisting of a basal (Difco, Bacto Yeast Nitrogen Base for Carbon Assimilation Tests) medium supplemented with citric acid, lactic acid or urea and combinations of these. The supplements were used at the 1% level of concentration. The pH of the medium was adjusted to 6.8 to 7.2 and sterilized by autoclaving.

The inocula consisted of a washed cell suspension of a 24-hour culture of the test organisms. Culture media were inoculated with 0.5 ml of cell suspension adjusted to give a percent transmittance of 10 which corresponded to approximately 4 mg of cells on a dry weight basis. Cultures were incubated at 25 to 28°C.

The ability of the test organisms to initiate growth on citrate and acetate with either urea or ammonium chloride as the sole cource of carbon and nitrogen, respectively was measured in terms of an increase in cell density corresponding to a decrease in percent light transmittance of the culture.

The bacterial species used in the study were: Aerobacter aerogenes, Proteus

vulgaris, Pseudomonas aeruginosa, and Serratia indica.

III. Respiration Studies:

The oxidation of citrate and lactate by resting cells of Pseudomonas aeruginosa and Serratia indica was measured manometrically by the method described by Umbreit et al., 1957.

The Warburg flasks usually contained 1.0ml of resting cell suspension of the test organsims and 1.0 ml of M/15 phosphate buffer (pH 7.0). The substrate was introduced from the side arm and unless otherwise stated consisted of 1 ml of 0.01 M solution. The center wll contained 0.2 ml of 10% NaOH and a filter-paper wick. The flasks were incubated at 30° C and equilibrated 30 minutes before tipping in the substrate. The manometer readings were made at 15 minute intervals for a 2-hour incubation period.

The data are reported as the total microliters of 0_2 uptake.

The utilization of urinary citric acid by bacteria was measured in terms of the percent reduction in the citric acid content of urine cultures as measured by the method of Snell and Snell, 1953. In addition, the corresponding growth of each of the organisms was determined at each citric acid measurement by the procedure heretofore described.

RESULTS AND DISCUSSION

The data in Table 1 show that Proteus vulgaris was able to reduce the urea content of urine by 26.09, 61.69, 88.68 and 96.11% for incubation periods of 0-12, 12-24, 24-48, and 48-72 hours, respectively. Table 2 shows that the overall reduction in the urea content of cultures over all trials was 94.72 to 95.27%.

Of the four organisms employed in the experiment, only P. vulgaris was observed to give a positive urease reaction.

Other organisms tested for their ability to convert urinary urea were unable to reduce the urea content by more than 22.92 to 37.10%. These values were not

considered to be significant when compared with those obtained with P. vulgaris.

The results of tests designed to evaluate the utilization of urinary urea by selected species of bacteria have clearly demonstrated that P. vulgaris is clearly the organism of choice. The other organisms employed appeared to have limited application for biological systems where urea will constitute the major source of nitrogen. For this reason, A. aerogenes and S. indica have been eliminated with respect to urea converison.

The data in Table 3 show that citric acid utilization (% reduction) and corresponding growth values (% transmittance), respectively amounted to: A. aerogenes-68.4, 31.6; P. vulgaris-66.77, 32.5; P. aeruginosa-77.32, 22.5 and S. indica-80.94, 19.0.

On synthetic media containing citrate as the sole source of carbon all the test organisms were able to inititate rapid and excellent growth (Figure 1).

These data indicated that the test organisms can utilize citric acid as a sole source of carbon and energy.

Table 4 shows that A. aerogenes and S. indica were able to produce excellent growth on a synthetic medium containing lactate and urea as sole source of carbon and nitrogen.

The results of tests designed to determine the growth of bacteria on a synthetic medium containing citrate and lactate as sole sources of carbon and energy have demonstrated that these substances can be used for initiating growth.

Data were obtained demonstrating that S. indica cells grown on yeast extract enriched nutrient broth were superior to those grown on urine enriched broth in their ability to grow on a synthetic medium containing citrate or lactate as the sole source of carbon and energy. This suggested that urine is deficient in essential metabolic precursors and/or vitamins.

Respiration studies have demonstrated that resting cells of Ps. aeruginosa

and S. indica were not capable of oxidizing citrate. This occurrence has been attributed to impermeability of the cell wall of the organisms to citrate . Lactate was observed to be oxidized rapidly and extensively. The extent of citrate and lactate oxidation, respectively in terms of 0_2 uptake was: Ps. aeruginosa-53, 108 and S. indica-40, 392.

The data in Table 5 show that when a combination of resting cells of Ps. aeruginosa and S. indica were used a considerable amount of 0_2 uptake was observed by the cells on the citrate substrate. This suggested that there exists a metabolic interrelationship between P. aeruginosa and S. indica for citrate oxidation.

Other tests run in connection with the oxidation studies indicated that the enzymes for lactate oxidation are adaptable in nature.

The respiration data indicated that S. indica would be the organism of choice for the efficient utilization of the lactic acid of human urine.

The results of the respiration studies have given us some insight into the nature of citrate and lactate utilization by the organisms employed in the study in relation to their potential usefulness on a urine substrate employed in biological systems intended for outer spaces.

Table 1. Reduction in urinary nitrogen by a growing culture of Proteus vulgaris.

Incubation hours	Duplicate cultures	Duplicate samples	TRIALS I O.D.** reading	TRIALS I Urea mg/ml	TRIALS II O.D.** reading	TRIALS II Urea mg/ml
0	A	1	0.535	19.87	0.475	23.15
		2	0.430	15.95	0.470	22.95
	B	1	0.470	17.44	0.420	20.45
		2	0.515	19.10	0.455	22.22
12	A	1	0.335	15.24	***	
		2	0.242	11.01		
	B	1	0.300	13.65	***	
		2	0.298	13.60		
24	A	1	0.188	5.26	0.335	15.24
		2	0.095	2.66	0.300	13.65
	B	1	0.080	2.24	0.242	11.01
		2	0.060	1.71	0.298	13.60
48	A	1	0.045	2.34	0.188	5.70
		2	0.030	1.56	0.188	5.70
	B	1	0.00	0.00	0.080	2.43
		2	0.00	0.00	0.060	1.83
72	A	1	0.032	1.10	0.045	1.18
		2	0.00	0.00	0.035	0.92
	B	1	***		***	
		2				

*** No analysis made
** Optical density

Table 2. Summary table of reduction in urinary nitrogen by growing cells of Proteus vulgaris

Incubation	Samples W/ cultures I A	Samples W/ cultures I B	Cultures W/ trials II A	Cultures W/ trials II B	Cultures W/ trials I A-B	Cultures W/ trials II A-B	Samples W/ cultures I A	Samples W/ cultures I B	Samples W/ cultures II A	Samples W/ cultures II B	Cultures W/ trials I A-B	Cultures W/ trials II A-B	Total urea reduction incubation
hours	mg/ml	mg/ml	mg/ml	mg/ml	mg/ml	mg/ml	%	%	%	%	%	%	%
0	17.91	18.27	23.05	21.33	18.09	22.19	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12	13.12	13.62	***	***	13.37	***	26.74	25.45	***	***	26.09	***	26.09
24	3.96	1.97	14.44	12.30	2.96	13.37	77.88	89.22	37.35	42.33	***	39.75	61.69
48	1.95	0.00	5.70	2.13	0.91	3.91	89.11	100.00	75.27	90.00	83.63	82.35	88.68
72	0.55	0.00	1.05	***	0.55	1.05	96.93	100.00	95.44	90.00	94.97	95.27	96.11

W/ - within

- over

*** - no analysis made

Table 3. The relative growth and citric acid utilization by cultures of A. aerogenes, P. vulgaris, Ps. aeruginosa and S. indica growing on a urine substrate

Incubation	Duplicate cultures	Aerobacter aerogenes Citric acid reduction	Growth		Proteus vulgaris Citric acid reduction	Growth		Pseudomonas aeroginosa Citric acid reduction	Growth		Serratia indica Citric acid reduction	Growth	
		O.D.	conc.**	Turbidity	O.D.	conc.**	Turbidity	O. D.	conc.**	Turbidity	O.D.	conc.**	Turbidity
hours	No.	Reading	mg/ml	%T	Reading	mg/ml	%T	Reading	mg/ml	%T	Reading	mg/ml	%T
0	1	*0.061	1097.7	98.0	0.061	1097.7	98.0	0.061	1097.7	98.0	0.061	1097.7	98.0
	2	*0.061	1097.7	98.0	0.061	1097.7	98.0	0.061	1097.7	98.0	0.061	1097.7	98.0
24	1	0.021	406.6	37.0	0.033	636.5	58.0	0.023	444.6	40.0	0.028	541.5	49.0
	2	0.015	290.7	26.0	0.033	636.5	58.0	0.015	290.7	26.0	0.015	290.7	26.0
48	1	0.023	300.2	27.0	0.028	366.7	33.0	0.018	235.6	21.5	0.017	222.6	20.0
	2	0.030	393.3	36.0	0.025	326.8	30.0	0.019	262.2	24.0	0.015	195.7	18.0
Total reduction (%) in citric acid			68.4			66.77			77.32			80.94	

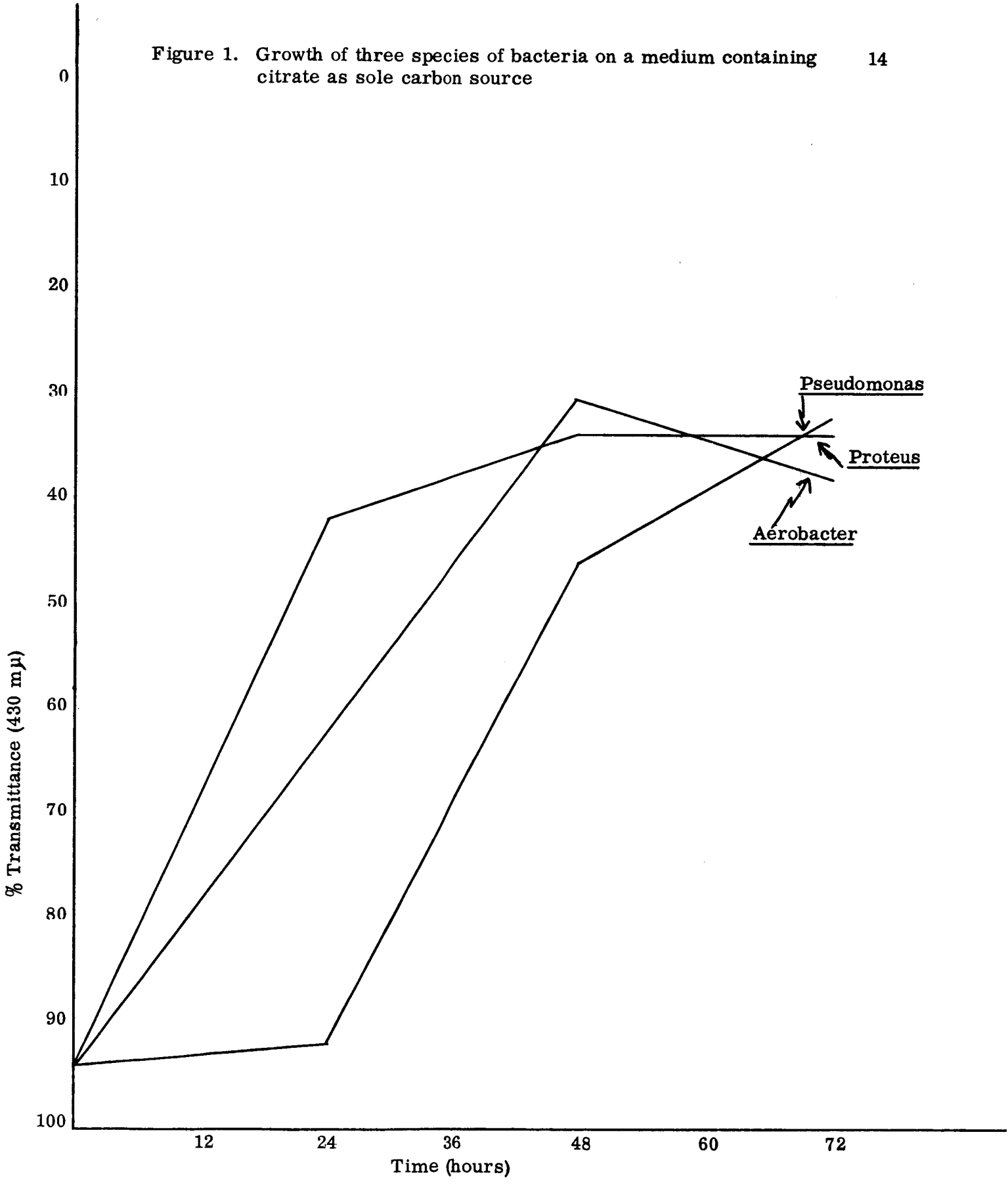
* Average for all cultures
** Concentration

Table 4. The growth of A. aerogenes and S. indica on a medium containing lactic acid and urea as sole sources of carbon and nitrogen

		SUBSTRATE					
		Mineral salts-Lactate-Ammonium Chloride		Mineral Salts-Glucose-Urea		Mineral Salts-Lactate-Urea	
		Turbidity					
	Incubation	Aero.	Serr.	Aero	Serr.	Aero.	Serr.
	hours	%T	%T	%T	%T	%T	%T
R-I	0	96.2	97.5	96.2	95.5	96.5	95.8
	12	96.0	97.0	95.5	95.2	83.0	95.5
	24	70.0	67.5	88.0	95.0	80.5	66.0
	48	61.0	51.2	78.5	64.9	82.0	43.5
	72	66.5	57.8	79.0	64.0	83.5	55.0
R-II	0	66.0	56.0	97.0	97.5	54.5	54.5
	12	55.5	50.2	83.0	74.6	41.5	54.0
	24	35.0	40.0	61.5	56.0	37.0	54.0
	48	23.0	13.0	53.0	36.0	11.0	**
	72	10.0	10.0	42.3	27.0	10.0	**

Aero. = Aerobacter aerogenes: Serr. = Serratia indica

Figure 1. Growth of three species of bacteria on a medium containing citrate as sole carbon source



PART II

1. Monosaccharide components of extracellular polysaccharides of seven species of algae.

The quantitative production of extracellular polysaccharides by selected species of algae has been reported in NASA Progress Report No. V. This study was to determine the constitutive monosaccharides in the polysaccharides.

METHODS AND PROCEDURES

Polysaccharides were hydrolyzed by the method of Lewin, 1956. Chromatographic procedures were taken from Block et al., 1958 and Glick, 1954.

RESULTS AND DISCUSSION

The monosaccharides in the polysaccharide hydrolysates are given in Table 1. The results show that various hexoses, pentoses, methyl-pentoses, and a uronic acid are present in the polysaccharides. Glucose was present in most of the polysaccharides and this is important from the viewpoint of energy-supply.

2. Biochemical characteristics and mechanisms of the extracellular polysaccharide of Palmellococcus.

The polysaccharide produced by Palmellococcus was selected for further study because of its production figures (NASA Report No. V).

a. Molar ratio of the constitutive monosaccharides of the extracellular polysaccharide.

To determine the amount of the various monosaccharides in the in the polymer, a molar ratio of the sugars was determined.

METHODS AND PROCEDURES

Hydrolysis was performed as in section 1. Chromatographic separation and elution were carried out according to Hough (1952) and Hawthorne (1947). Reducing sugar measurements were made by the Somogyi method (Nelson, 1944).

RESULTS AND DISCUSSION

The extracellular polysaccharide of Palmellococcus exhibited a molar ratio of glucose:arabinose:fucose:glucurone as 11:3:6:1. Thus, the microorganism can furnish 3 sugars in high proportions which are usable by other organisms.

b. Studies on the biosynthesis of the extracellular polysaccharide of Palmellococcus.

No synthetic pathway for algal polysaccharides of extracellular nature has been described. It was the object of these experiments to furnish data which would give preliminary conclusions concerning the probable route of extracellular polysaccharide synthesis by green algae.

METHODS AND PROCEDURES

Palmellococcus cells were exposed to radioactive carbon sources in an illuminated chamber for varying time periods. Polysaccharide fractions (extracellular, cellular, and intracellular) were extracted with absolute ethanol. Hydrolysis procedures were the same as described in section 1. Radioactivity measurements were made with a thin-window Geiger tube. Corrections were made for backgroud only to determine the relative uptakes.

Cells were exposed to $NaHC^{14}O_3$ to determine the time required to label the extracellular polysaccharide. Grown cells and growing cells were exposed to $NaHC^{14}O_3$ and glucose $-C^{14}$ (ul) to determine the amount and rate of polysaccharide uptake. Long-time exposure to $Na\ HC^{14}O_3$, glucose-C^{14} (ul), and formate-C^{14} was tested for concentration of the radioactivity in the polysaccharides.

RESULTS AND DISCUSSION

It was found that C^{14} was incorporated into the extracellular polysaccharide between 80 minutes and 24 hours. With grown cells and 12 hours exposure, the results show that glucose is incorporated into the polysaccharides very fast while

bicarbonate-C^{14} is very slow. In 24 hours with grown cells, there was little difference. Growing cells gave essentially the same results but a high uptake was not achieved. In long-time exposure, the C^{14} was concentrated in the polysaccharides only with glucose -C^{14}.

The extracellular, cellular, and intracellular polysaccharides were shown to be the same in composition. From the rapid incorporation of glucose-C^{14} into the polysaccharides, it is evident that this hexose may play an important role in the synthesis of algal polysaccharides.

c. Extracellular polysaccharide production by Palmellococcus with various carbon precursors and nitrogen sources.

Palmellococcus was grown on various carbon and nitrogen sources to determine qualitative and quantitative effects upon the extracellular polysaccharide.

METHODS AND PROCEDURES

Algal cell dry weights were determined by difference at 90°C. Extracellular polysaccharide in culture filtrates was determined by the anthrone method of Matton et al., 1955. Chromatographic analysis was followed as in section 1.

RESULTS AND DISCUSSION

Glucose and lactate polysaccharides exhibited extra sugars over the 4 found in the CO_2 polysaccharide. These were 2-deoxyribose, xylose, and an unknown sugar. Acetate, pyruvate and glyceraldehyde polysaccharides were absent one sugar, glucurone. Formate and bicarbonate polysaccharides gave the same sugars as the CO_2 polysaccharide. Only glucose gave more polysaccharide in the culture filtrate than CO_2. The others were very much lower. Dry weight followed the same trend.

The nitrogen sources tested ($CaNO_3$, KNO_3, NH_4NO_3, urea, creatinine and $NaNO_3$) gave results which showed that $CaNO_3$, NH_4NO_4, urea, and $NaNO_3$ are better suited nitrogen sources for polysaccharide production than the standard

nitrogen source, KNO_3. Dry weight measurements showed that only $CaNO_3$ and urea were better nitrogen sources than KNO_3.

3. Growth and Polysaccharide Production by Palmellococcus Grown on Waste Substrates.

These experiments were to determine if algal cells could feasibly be grown on a waste substrate and at the same time enrich the substrate with cellular organics.

METHODS AND PROCEDURES

Dry weights and extracellular polysaccharide in culture filtrates were determined as in previous sections. The waste substrate was an electrolyzed solution of 15 parts of urine to one part of feces.

RESULTS AND DISCUSSION

Growth on the waste substrates was very good (1.2 gram-cell/liter). Extracellular polysaccharide production was very low even when the medium was supplemented with nitrogen. Total carbohydrate data showed that the substrates could be enriched with cellular organics to a great extent by algae.

4. Closed Ecology Energy Supply Studies

It has been found in the course of research on this project that the one limiting factor in producing a microbial food from waste is energy. The following experimental results exhibit forms, ways, and mechanisms of converting waste materials to potential energy supply systems for a closed ecology for long-space flights.

a. Carbohydrate analysis of Chlorella vulgaris with energy calculations.

These analyses were made to determine as exactly as possible the amount and type of carbohydrate compounds produced by a typical species of green algae.

METHODS AND PROCEDURES

Ch. vulgaris cells were separated into various carbohydrate fractions. Polymers were hydrolyzed as described in section 1. Reducing sugars were determined by the Somogyi method (Nelson, 1944) using glucose as the standard. Energy values were calculated using the caloric value of glucose.

RESULTS AND DISCUSSION

The results are displayed in Table 2. The results show that most of the carbohydrate and energy is bound up in non-extractable polysaccharides.

b. Utilization of algal polysaccharides (extracellular) as energy sources for Torula utilis.

These studies were performed to determine to what extent an organism such as Torula utilis can utilize the algal polysaccharides for energy.

METHODS AND PROCEDURES

Twenty mg quantities of the algal polysaccharides (seven algae reported in NASA Report No. V) were hydrolyzed, neutralized, and tested for exygen uptake by T. utilis. Unhydrolyzed polysaccharides were also tested for oxygen uptake. Standard manometric techniques from Umbreit et al., (1957) were followed.

RESULTS AND DISCUSSION

Hydrolyzed algal polysaccharides gave responses which varied with the species of algae. Palmellococcus and Chlamydomonas gave responses of 318 and 358 $\mu l\text{-}O_2$ uptake in 120 minutes. The other polysaccharides gave responses somewhat lower. Chlorella sp. and Ch. vulgaris gave very low responses, 69 and 35 $\mu l\text{-}O_2$ uptake in 120 minutes respectively.

Unhydrolyzed polysaccharides gave responses which on the average were one-fourth the response gained on the depolymerized polysaccharides.

c. Conversion of detergent-like compounds to carbohydrate material.

An organism has been isolated in this lab which converts the sulfonated higher alcohols to a water-soluble, extracellular polysaccharide containing glucose, arabinose, ribose and glucuronic acid. Since a source of the detergent compounds would be available in the waste of a spaceship, this is still another method of regenerating microbial energy.

METHODS AND PROCEDURES

The polysaccharide material was isolated from the growth media by alcoholic precipitation. Reducing sugar measurements were made by the Somogyi method (Nelson, 1944) on polysaccharide hydrolysates. Substrates tested for polysaccharide production were a mineral medium supplemented with various concentrations of Tide and sodium lauryl sulfate and nutrient broth supplemented with Tide and sodium lauryl sulfate. Polysaccharide from nutrient broth + 1% Tide was isolated and tested for the oxidative response of T. utilis using standard respirometric techniques.

RESULTS AND DISCUSSION

The results of polysaccharide production in terms of free reducing sugars in various substrates are given in Table 3. It can readily be observed that the conversion to carbohydrate is influenced by whether or not a protein source is present. The data also suggest that the polysaccharide is produced in sufficient quantities to require further investigation. The response of T. utilis to the polymer was 1080 μl-0_2 uptake in 90 minutes. Endogenous 0_2 uptake was 20 μ l-0_2/90 minutes. Oxygen uptake on a sugar solution containing 800 μg of glucose was 90 μl-0_2/90 minutes. This indicates that the polysaccharides can readily be oxidized by the yeast culture for energy.

d. Algal extracts as carbon-energy sources for Torula utilis.

The objectives of this research were to obtain a method for preparing algal extracts, to test the algal extracts of carbohydrates, to test the growth

response of a selected species of yeast on the algal extract, and to determine the oxidation of algal extracts by a species of yeast.

METHODS AND PROCEDURES

Algal extracts were prepared by exposing the algal cells to sonic vibrations in a 10KC Raytheon sonic oscillator. Dry weights, packed cell volumes, anthrone carbohydrate, chromatographic analyses, and oxygen uptake were determined by standard techniques.

RESULTS AND DISCUSSION

Packed cell volumes, soluble carbohydrate, and dry weight determinations showed that sonification was a quick and efficient method of disrupting algal cells. The extent of solubilization of the algae varys with the cell concentration as expected. With cell solutions of less than 3mg/ml, over 90% reduction in dry weight can be achieved in 15 minutes exposure.

Sonification of Palmellococcus releases two types of carbohydrates; (1) free sugars as glucose, arabinose and fucose, and (2) bound sugars composed of glucose, mannose, arabinose, ribose, fucose and glucurone.

The use of the extract of Palmellococcus cells as a growth medium for T. utilis gave very encouraging results. The population of yeast cells increased by a factor of 40 (1.9×10^5/ml to 8.0×10^6/ml) in 24 hours. The control flask with no carbon and whole algal cells did not change to any extent over the initial inoculum.

The oxidation of the algal extracts gave results which are an indication of T. utilis's ability to use the algal extracts as energy sources.

Oxygen uptake vs. time of soneration (Figure 1) shows that the maximum response is gained when the algae are sonerated for 45 minutes. This difference is not large, however, and would be impractical for technical application.

Supplementing the algal extracts with glucose and peptone increased the oxidative response of the yeast but not to a large extent (Figure 2). With protein

and chlorophyll removed by lead acetate and barium oxalate, a response was gained which was better than the algal extract with and without supplementation. This medium was also perfectly clear after the protein and chlorophyll removal.

From these results, the role of algae would seem to be more of energy supply than as a source of carbon in a closed ecology.

Table 1. Monosaccharides in algal extracellular polysaccharide hydrolysates

Algal Species	Spot on Chromatogram		Phenol Rf*	Butanol Rf*	Monosaccharide
Palmellococcus sp.	1		0.3395	0.1946	Glucose
	2		0.4383	0.2373	Arabinose
	3		0.5638	0.3027	Fucose
	4		0.7043	0.3520	Glucurone
Chlorella sp.	1		0.3534	0.2427	Glucose
	2		0.4942	0.2880	Xylose
	3		0.6014	0.3747	Rhamnose
	4	(trace)	0.7195	0.2854	Glucurone
Chlorella ellipsoidea	1		0.3138	0.2067	Glucose
	2		0.4512	0.2467	Arabinose
	3		0.5925	0.3333	Fucose
	4	(trace)	0.7848	0.3579	Glucurone
Anabena sp.	1		0.3390	0.1986	Glucose
	2		0.4638	0.2480	Arabinose
	3		0.5678	0.3280	Fucose
	4		0.6860	0.3107	Glucurone
Chlorella vulgaris	1		0.3160	0.1958	Glucose
	2		0.4480	0.2785	Xylose
	3		0.5900	0.4238	Rhamnose
Chlamydomonas sp.	1		0.3222	0.2334	Glucose
	2		0.4490	0.2938	Xylose
	3		0.5647	0.3720	Fucose
Oocystis	1		0.3850	0.2347	Galactose
	2		0.4971	0.2906	Arabinose
	3		0.5808	0.3333	Fucose
	4	(trace)	0.6959	0.3146	Glucurone

*RF's are averages of triplicate chromatograms. NOTE: Glucurone, the lactone of glucuronic acid, was found in most of the hydrolysates. Lactone formation takes place in acid solutions and this is the explanation of why the lactone instead of the uronic acid was obtained.

Table 2. Carbohydrate analysis of Chlorella vulgaris

Sample	Total Carbohydrate as Glucose per 1456 mg algae	% of Cell Weight	Energy
	mg	%	Kcal
FRS in Medium	0.00	0.00	------
FRS in Extracellular Polysaccharide Hydrolysate	10.02	0.688	0.03747
FRS in Alcoholic Extract of Cells	0.699	0.05	0.0026
FRS in Sonic Extract of Cells	3.699	0.25	0.0138
FRS in Intracellular Polysaccharide Hydrolysate	14.732	1.01	0.0551
FRS in Hydrolysate of Residual Cells	93.110	6.39	0.3482
Total FRS = 4.398 mg		0.302	
Total Bound Sugars as FRS = 117.862		8.095	
Total Carbohydrate as Glucose = 122.260		8.397	

Total Carbohydrate energy: 0.457 Kcal/1456 mg = 0.315 Kcal/gram
FRS = Free Reducing Sugar

Table 3. Quantitative polysaccharide production in a mineral medium and nutrient broth supplemented with sodium lauryl sulfate and Tide

Medium	% Tide	% Sodium Lauryl Sulfate	Polysaccharide in substrate as glucose
	%	%	µg/ml
Nutrient Broth	0	0	11.24
Nutrient Broth		1.0	36.00
Nutrient Broth		2.0	179.35
Nutrient Broth	1.0		111.89
Mineral Medium	1.0		7.90
Mineral Medium	1.5		11.20
Mineral Medium	2.0		11.95
Mineral Medium		1.0	10.65
Mineral Medium		1.5	12.65
Mineral Medium		2.0	3.16

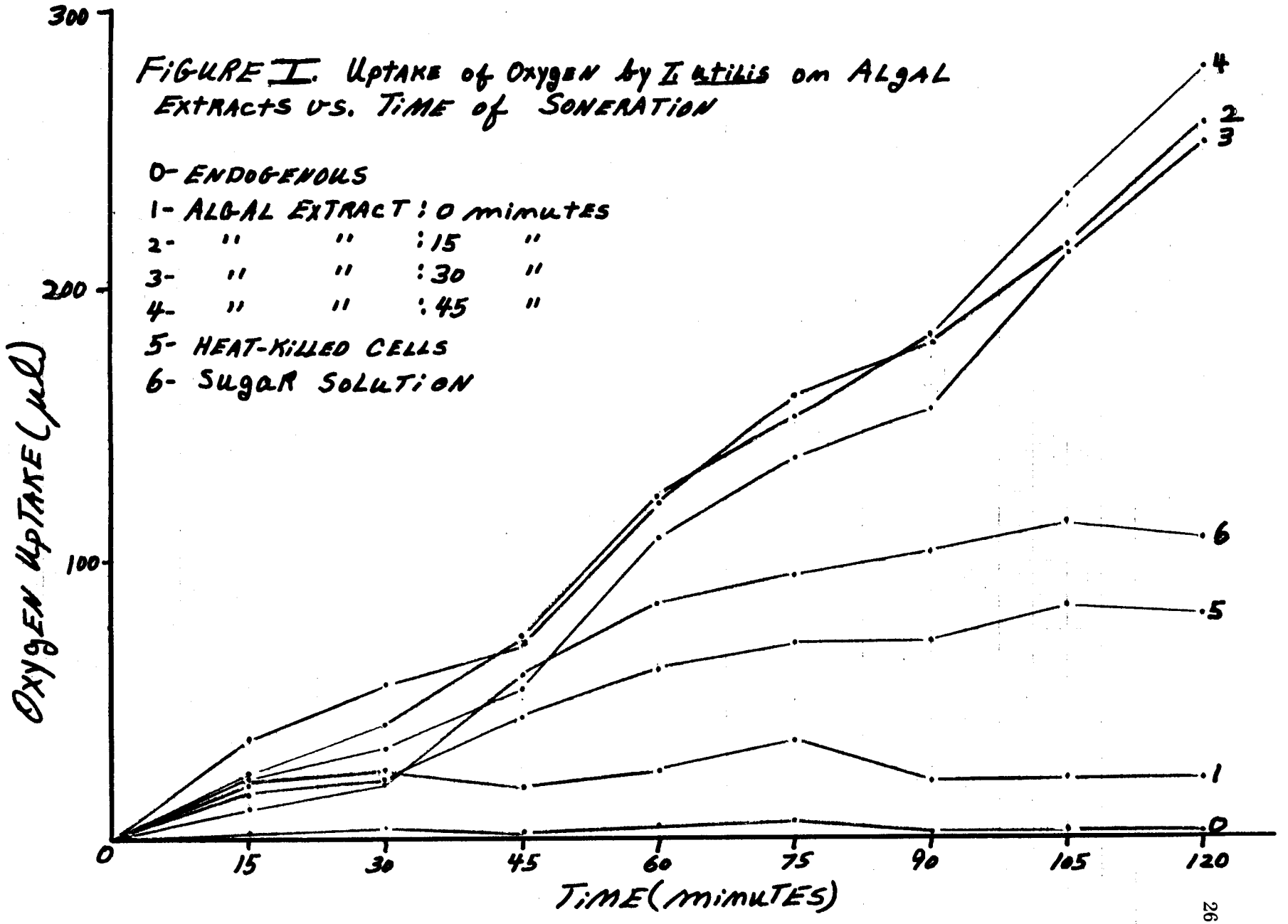


FIGURE I. Uptake of Oxygen by I. utilis on Algal Extracts vs. Time of Soneration
0- Endogenous
1- Algal Extract : 0 minutes
2- " " : 15 "
3- " " : 30 "
4- " " : 45 "
5- Heat-killed cells
6- Sugar Solution
Oxygen Uptake (µl)
300
200
100
0
15
30
45
60
75
90
105
120
Time (minutes)
4
2
3
6
5
1
0

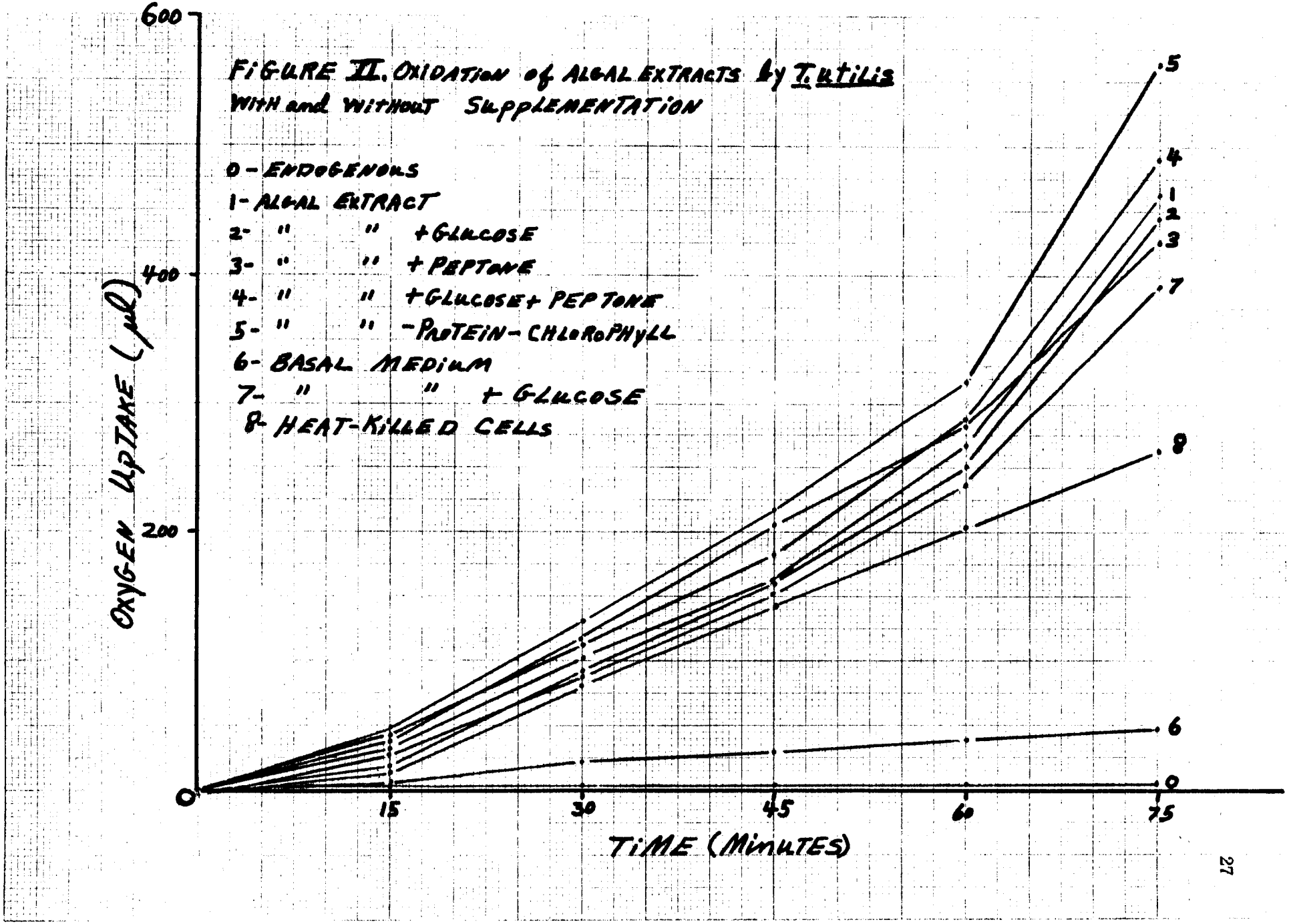


FIGURE II. OXIDATION of ALGAL EXTRACTS by T. utilis
WITH and WITHOUT SUPPLEMENTATION
0 - ENDOGENOUS
1 - ALGAL EXTRACT
2 - " " + GLUCOSE
3 - " " + PEPTONE
4 - " " + GLUCOSE + PEPTONE
5 - " " - PROTEIN - CHLOROPHYLL
6 - BASAL MEDIUM
7 - " " + GLUCOSE
8 - HEAT-KILLED CELLS
OXYGEN UPTAKE (µl)
600
400
200
0
15
30
45
60
75
TIME (Minutes)

FUTURE RESEARCH

Research efforts planned for future investigation are to be concentrated on the supply of energy to the closed ecological system.

Algal carbohydrates as a source of energy will be further investigated. The pathways of biosynthesis of energy-rich compounds of algal origin will be elucidated.

The conversion of detergent-like compounds to carbohydrate will be further investigated.

Other feasible methods of supplying microbial energy to a closed ecological system will be investigated.

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